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ABSTRACT

Incidence of bone metastases is very high in advanced prostate cancer patients. Bone metastases likely have a significant impact on functional status and quality of life, not only related to pain, but also to the relevant risk of skeletal-related events. A better understanding of mechanisms associated with bone metastatic disease secondary to prostate cancer and more specifically to the cross-talk between tumor cells and bone microenvironment in metastatic progression represented the background for the development of new effective bone-targeted therapies. Furthermore, a better knowledge of biological mechanisms driving disease progression led to significant advances in the treatment of castration-resistant prostate cancer, with the development and approval of new effective drugs. Aim of this review is to outline the physiopathology of bone metastases in prostate cancer and summarize the main results of clinical trials conducted with different drugs to control morbidity induced by skeletal metastases and bone disease progression. For each agent, therapeutic effect on bone metastases has been measured in terms of pain control and/or incidence of skeletal-related events, usually defined as a composite endpoint, including the need for local treatment (radiation therapy or surgery), spinal cord compression, pathological bone fractures. In details, data obtained with chemotherapy (mitoxantrone, docetaxel, cabazitaxel), new generation hormonal agents (abiraterone, enzalutamide), radium-223, bone-targeted agents (zoledronic acid, denosumab) and with several experimental agents (cabozantinib, dasatinib, anti-endothelin and other agents) in patients with castration-resistant prostate cancer are reviewed.

KEYWORDS

Castration resistant prostate cancer, bone metastasis, skeletal related event, new generation hormonal agents, chemotherapy, bone-targeted therapy

INTRODUCTION

In developed countries, prostate cancer is the most common type of cancer diagnosed among men, with more than 1,100,000 new cases worldwide every year [1]. In this cancer, bone represents a preferential site of metastases, and patients with advanced disease have a very high incidence of bone metastases [2]. Autopsy data from prostate cancer patients indicate an incidence of secondary bone lesions as high as 65%-75%, preceded only by multiple myeloma [3]. These bone metastases are typically osteosclerotic (i.e. with increased osteoblastic activity), and likely to produce a significant impact on patients' functional status and quality of life (QoL), not only related to pain, but also to the relevant risk of skeletal-related events (SREs) that can negatively impact physical well-being and activities of daily living [4,5]. According to Food and Drug Administration [6], skeletal related events (SREs) include pathologic bone fractures (both vertebral and non-vertebral), spinal cord compression, surgery to bone, radiotherapy to bone. To estimate the incidence of SREs in patients with prostate cancer and bone metastases the control arm of the trials testing bisphosphonates may be used as a reference value [7]. In a 15-month observation period, nearly half (44.2%) of those patients experienced at least one SRE.

SREs may have a relevant impact on survival of prostate cancer patients with bone metastases. In a landmark analysis of a randomized trial comparing zoledronic acid (ZA) versus placebo, patients without SREs in the first six months had significantly better 1-year survival rate compared to patients suffering from one or more SRE [8]. Furthermore, survival of patients with multiple events was worse than propensity-matched patients with only one SRE, although this difference was not statistically significant. A secondary analysis of randomized trials with ZA showed that, in patients with metastatic prostate cancer similarly to other tumor types, the incidence of pathological fractures is associated with a significantly increased risk of death [7]. In details, patients with pathological

fractures had a 29% increase in the risk of death at the unadjusted analysis (Hazard Ratio [HR] 1.29, 95% confidence interval [CI] 1.01-1.65), with comparable results observed for both vertebral and non-vertebral fractures. Adjusted analyses for prognostic covariates, including previous SRE occurrence and performance status, led to comparable results. As expected, although prostate cancer patients with metastatic spinal cord compression had a relatively better life expectancy compared to other tumors, this complication has a relevant impact on survival [9,10].

Patients with a SRE have a significantly worse QoL [5,8] and when assessed by validated instruments, such as the Functional Assessment of Cancer Therapy-General (FACT-G) and the Brief Pain Inventory (BPI), a clearly worse outcome was observed in patients with SREs compared to those without, with statistically significant differences in FACT-G total score, in functional well-being, physical well-being, emotional well-being and in BPI score [8]. When all types of SRE were considered as a whole (need for radiation, pathological fractures, other SRE) there was a statistically significant and clinically relevant decline in QoL in all domains [5]. Of course, treatment of SRE can improve QoL: radiation therapy can produce a significant reduction of pain [5], while treatment of spinal cord compression may improve performance status [10]. The occurrence of bone complications is also likely be responsible of the increased direct and indirect costs of patients' management [11].

All SREs are associated with relevant health resource utilization, including both inpatient hospitalizations and outpatient or emergency department visits and procedures [12-14]. Furthermore, those studies trying to calculate the costs associated with SREs may have under-estimated their global impact in terms of health resource utilization, due to the exclusion of patients with low performance status or life expectancy, and the exclusion of resource consumption associated with bone pain management [15].

Recently, the management of castration-resistant prostate cancer (CRPC) significantly changed, with approval of several new drugs [16]. This evolving therapeutic landscape was paired by a better knowledge of biological mechanisms driving disease progression. Nowadays, we know that AR signalling pathway has a significant activity also in CRPC and that the interplay between prostate cancer cells and bone microenvironment plays a crucial role in bone metastatic progression.

Aim of this review is to outline the physiopathology of bone metastases in prostate cancer and the contribution of each of these new agents in terms of control of morbidity induced by skeletal metastases and bone disease progression.

PHYSIOPATHOLOGY OF BONE METASTASES IN PROSTATE CANCER

Bone metastasis is a complex event due to the interaction among cancer cells, normal bone cells and bone microenvironment, leading to a severe disruption of physiological bone remodeling [17]. The latter is a dynamic process, critical to maintain skeletal integrity, responsible for replacement of old bone with a mechanically more competent bone. It occurs at specialized skeleton sites - called “bone remodeling units” - and is characterized by a functional sequence: osteoclast-mediated bone resorption followed by osteoblast-induced bone apposition [18,19].

In the early phase of bone remodeling, osteoclasts are attracted to bone surface, in which these cells excavate the Howship’s lacuna, a resorption cavity. Following the cavity formation, osteoclasts produce several factors responsible for osteoblasts attraction to the sites of previous resorption. This sequence of events is called “coupling phenomenon”. As described by Paget in 1889, tumor cells are “the seeds” which need a favorable “soil” in order to thrive at metastatic sites [20]. Skeletal microenvironment is an ideal “soil”, due to presence of growth factors and cytokines stored in the bone matrix and released during cross-talk between bone-resident cells and cancer cells [21].

In the metastatic cascade, the first step is the homing of tumor cells to skeletal tissue [21,22]. This process is not a casual event, but is due to the production by bone microenvironment of the same chemotactic factors responsible for the migration of hematopoietic stem cells into the bone marrow. These cells are localized at a specific site, the hematopoietic stem cell niche, where they may remain quiescent or divide and then differentiate. An important chemotactic factor is the stromal-derived factor-1 (SDF-1), also called CXCL12. This cytokine, mainly produced by osteoblasts, interacts with the CXCR4 receptor on hematopoietic stem cells, inducing their homing to the bone marrow [21-24]. The pathway SDF-1/CXCR4 is also able to modulate the attraction of prostatic tumor cells to bone. Some preclinical studies showed a significant expression of CXCR4 on the

surface of prostate cancer cells [25]. The induction of SDF-1 expression from bone marrow endothelial cells favors prostatic cancer migration and adhesion to extracellular bone matrix [22,26]. Therefore, prostatic tumor cells are able to compete with hematopoietic stem cells for the place in the bone marrow niche; this complex process determines the formation of so-called “onco-niche”, in which cancer cell may remain in a state of dormancy or may start to colonize and invade (**Figure A**) [17,21,22].

During the metastatic colonization of the bone, prostate cancer cells interfere with the physiological bone remodeling due to the release of paracrine factors physiologically involved in the regulation of both osteoclastic and osteoblastic activity (**Figure B**). The early, crucial phase of this process is the abnormal increase of osteoclast-mediated bone resorption, due to several growth factors and cytokines, as transforming growth factor β1 (TGF β1), parathyroid-hormone-related peptide and interleukin 6 [27]. These factors lead to the activation of the receptor activator nuclear kappa B (RANK) / RANK ligand (RANKL) pathway, which plays a central role in bone resorption regulation. RANKL, produced by osteoblasts, binds its receptor RANK on osteoclasts surface, favoring osteoclast maturation, survival and activity [17,21]. Increased osteolysis is crucial for the seeding of prostate cancer cells, and is also associated with the release from the bone matrix of several growth and survival factors, responsible for tumor progression [27]. In the subsequent phase of skeletal colonization there is an excessive bone apposition, which becomes dominant compared to bone resorption. This is due to growth factors including basic fibroblast growth factor, bone morphogenic proteins, endothelin-1 (ET-1), tumour growth factor β1 and insulin-like growth factor 1, that are released by cancer cells and from bone matrix and stimulate both osteoblasts activity and tumor proliferation. Prostatic cancer cells may also contribute to bone apposition by gaining the same functional activities of osteoblasts (“osteomimicry”) [27].

The complex interaction between bone microenvironment and tumor cells leads to the so-called “vicious cycle”, that induces cancer progression [17].

Prostate cancer patients with bone metastases frequently have SREs due to increased osteolysis in typically osteoblastic bone lesions [17,28]. Increased osteoclastic activity is not only confined to metastatic sites, but it may be considered a more generalized event [17,28]. This is caused by secondary hyperparathyroidism, due to the so-called “bone hunger syndrome”, a metabolic derangement in which calcium entrapment in skeletal tissue, due to increased osteoblastic activity, leads to hyperparathyroidism in response to serum calcium deficiency [29]. Compensatory increase of parathyroid hormone secretion is responsible of osteoclasts activation at distant sites.

Furthermore, an additional cause of bone resorption is represented by iatrogenic osteoporosis, induced by androgen deprivation treatment [28].

Skeletal related events: different definitions.

In older trials, therapeutic effect on bone metastases was measured in terms of pain, decrease in biochemical markers of bone turnover, serial imaging assessment showing healing of bone lesions [30]. In recent trials, SREs have been defined as a composite endpoint, mostly including the need for local treatment (radiotherapy or surgery), spinal cord compression and pathological bone fractures [31-36]. Radiotherapy may include treatment of uncontrolled pain, treatment or prevention of imminent pathologic fractures, treatment or prevention of spinal cord compression. Surgery may include procedures to stabilize pathologic fractures or spinal cord compression, but also procedures aimed to prevent these SREs. Some trials consider only skeletal symptomatic events (SSE), other trials include also asymptomatic bone fractures. Only in some trials the use of radioisotopes is explicitly included among the radiation therapy procedures. **Table A** summarizes the definition of SREs in selected randomized trials conducted in patients with metastatic prostate cancer, using SREs as primary or secondary endpoint.

IMPACT OF ANTI-CANCER TREATMENTS

Chemotherapy

Mitoxantrone -

At the beginning of this century, mitoxantrone plus prednisone was commonly used in CRPC patients for its palliative role, despite the negative outcome of randomized trials that did not show a significant improvement in overall survival (OS) [37,38]. In one trial CRPC patients with pain received mitoxantrone plus prednisone or prednisone alone (**Table B**) [37]. Most of the enrolled patients (96%) had bone metastases. The primary endpoint was palliative response, defined as pain decrease without an increase in analgesics use. Palliative response rate was 29% with mitoxantrone plus prednisone and 12% with prednisone alone (p=0.01). Decrease in analgesics use without an increase in pain, one of the secondary endpoints, was comparable in the two arms. Later, another trial compared hydrocortisone alone vs. hydrocortisone plus mitoxantrone (**Table B**) [38]. Although there was no significant OS benefit, which was the primary endpoint, frequency and severity of pain were significantly better with mitoxantrone. Unfortunately, none of the trials included a description of SREs.

Docetaxel -

Before the TAX-327 [39] and the SWOG 99-16 study [40], that demonstrated the efficacy of docetaxel, no OS benefit had been shown with chemotherapy in CRPC patients. Both those two trials had OS as primary endpoint, while the impact on pain was among secondary endpoints (**Table B**). TAX-327 study compared two docetaxel schedules (every-3-week or weekly) plus prednisone versus mitoxantrone plus prednisone, and showed a significant OS benefit with every-3-week docetaxel [39,41]. Most patients (91%) had bone metastases and 45% had baseline pain. A reduction in pain was more frequently documented with every-3-week docetaxel than with mitoxantrone [39]. Pain response was

associated with OS outcome: median survival was 18.6 months among patients who achieved a pain response versus 12.5 months in patients who did not obtain pain response. However, improvement in median OS with every-3-week docetaxel was 3.9 months among men without significant baseline pain, and 2.4 months among those with baseline pain, suggesting that OS benefit associated with docetaxel is not limited to symptomatic patients obtaining pain response [42,43]. In the SWOG 99-16 trial, patients were randomized to docetaxel plus estramustine versus mitoxantrone plus prednisone [40]. Patients in docetaxel-estramustine arm had a significant OS improvement, although pain relief was similar in the two arms. In both these randomized trials, no specific SREs description was available.

Although not referred to CRPC but conducted in the "earlier" setting of hormone-naïve prostate cancer patients, in the STAMPEDE trial, the addition of docetaxel to androgen deprivation treatment (ADT) produced not only a relevant OS benefit (HR 0.78; p=0.006), but also a significant reduction in the time to first reported SSE (HR 0.60; p= 0.13 x 10^{-5}) ~~(Table B)~~ [44].

Cabazitaxel -

In preclinical and clinical models, cabazitaxel showed significant efficacy in docetaxel-resistant and refractory prostate carcinomas [45,46]. In the randomized phase III TROPIC trial, comparing cabazitaxel plus prednisone versus mitoxantrone plus prednisone in patients with metastatic CRPC after docetaxel failure, cabazitaxel was associated with a significant prolongation of OS [47]. More than 80% of patients had bone metastases, and about 45% had baseline pain. Secondary endpoints included pain response and time to pain progression, and cabazitaxel showed similar pain improvement compared to mitoxantrone (**Table B**) [48]. In an expanded access program conducted in United Kingdom, 31%-57% of patients treated with cabazitaxel reported "no pain or discomfort"

during treatment at various cycles, compared to 22% at baseline [49]. No specific description of the impact on SREs of cabazitaxel is available.

New generation hormonal agents

Abiraterone -

Abiraterone acetate (AA) is a potent, selective and irreversible inhibitor of CYP17, a critical enzyme in androgens synthesis [50]. The randomized trial COU-AA-301 compared AA plus prednisone vs. placebo plus prednisone in patients with metastatic CRPC (mCRPC) progressing after chemotherapy [51]. AA plus prednisone demonstrated a significant survival benefit [51,52]. At baseline, about 90% of patients in both arms had bone metastases, with similar pain scores. Incidence of SREs was 29% with AA and 33% with placebo; time to first SRE was significantly longer with AA (median 25.0 vs. 20.3 months; HR 0.615; p=0.0001) [35] (**Table B**). The most common SRE (expressed as rate per 100 patients-years of exposure) was bone radiation (24% with AA vs. 46.1% with placebo); others included pathologic fracture (6.0% vs. 4.0%), bone surgery (1.7% vs. 1.0%), and spinal cord compression (7.3% vs. 14.0%). In patients with clinically significant pain at baseline, AA produced significantly more palliation (45.0% vs. 28.8%; p=0.0005) and faster palliation of pain intensity (median time to palliation 5.6 vs. 13.7 months; p=0.0018) [35]. Iuliani and al. investigated AA activity on bone microenvironment in an *in vitro* model and in a clinical prospective cohort of 49 mCRPC patients, in which serum markers of bone turnover (ALP and CTX) were measured at baseline and every 3 months during treatment with AA [53]. AA was associated with a statistically significant inhibition of osteoclast differentiation and with osteoblasts differentiation. During treatment, patients had a progressive CTX reduction along with an increase of ALP values. In conclusion, this study demonstrated a direct bone anabolic and anti-resorptive effect of AA.

The randomized trial COU-AA-302 evaluated AA with prednisone compared to placebo plus prednisone in asymptomatic or mildly symptomatic mCRPC docetaxel-naive patients (**Table B**) [54]. Co-primary endpoints included radiographic progression-free survival (rPFS) and OS. The proportion of patients with bone disease only (51% and 49% in

experimental and control arm, respectively), and that of patients with more than 10 bone lesions (49% and 47%, respectively), were similar in the two arms. AA improved both OS and rPFS. Furthermore, secondary endpoints, such as time to symptomatic deterioration, time to pain progression and PSA PFS were significantly improved. Treatment with AA was associated with a significant improvement in time to opiate use (median not reached vs. 23.7 months; p=0.001), in time to increase in pain (median 26.7 vs. 18.4 months, p=0.049), and in time to progression of pain interference (median 10.3 vs. 7.4 months; p=0.005). Unfortunately, no data are available about the impact of treatments on SREs occurrence. A *post hoc* analysis evaluated the safety and efficacy of AA with concomitant bone targeted therapies (BTT) [55]. Overall, 34% of patients in experimental arm and 31% in control arm received concomitant BTT. Superiority of AA was confirmed both with and without BTT. Furthermore, although the interpretation of these results is limited by their *post hoc* nature, concomitant BTT prolonged time to opioid use (HR 0.80; p=0.036), time to performance status deterioration (HR 0.75; p<0.001) and was associated with better OS (HR 0.75; p=0.01). In a retrospective study of mCRPC patients treated with AA, out of 123 patients with baseline pain, 29% reported an improvement during treatment, 32% no change and 28% a worsening [56].

Enzalutamide -

Enzalutamide is an AR antagonist, more potent than first-generation drugs [57]. Similarly to abiraterone, enzalutamide is approved for the treatment of both patients with mCRPC progressing after chemotherapy and chemotherapy-naive patients. The AFFIRM phase III trial randomized men with mCRPC progressing after chemotherapy to enzalutamide *versus* placebo (**Table B**) [58]. At baseline, proportion of patients with bone lesions (about 92%), proportion of patients with more than 20 lesions (38%), and intensity of pain were similar between arms. Enzalutamide demonstrated a significant improvement in OS which

was the primary end point of the study, and its superiority was confirmed in all secondary endpoints. In details, median time to first SRE was 16.7 months with enzalutamide versus 13.3 months with placebo (HR 0.69; p<0.001) (**Table B**) [34]. Approximately half of patients were receiving a bisphosphonate at baseline. Time to first SRE was significantly improved by enzalutamide in patients not receiving bisphosphonate (HR 0.614; p=0.0005) and not significantly in patients who were receiving bisphosphonate (HR 0.762; p=0.553), although the study was not designed and powered to test this interaction. Enzalutamide provided consistent benefits in several pain measures, including pain severity, pain interference and pain palliation. Pain palliation was achieved in 45% of patients with enzalutamide versus 7% with placebo (p=0.0079).

The phase III PREVAIL study compared enzalutamide versus placebo in asymptomatic or mildly symptomatic patients with chemotherapy-naïve mCRPC (**Table B**), having OS and rPFS as co-primary endpoints [59]. Both were significantly improved with enzalutamide. At baseline, number of bone lesions and pain intensity were similar between arms. Although median time to first SRE was similar in the two arms, the risk of first SRE was significantly decreased with enzalutamide (HR 0.72; 95%CI 0.61-0.84; p<0.001) (**Table B**) [60]. Median time to pain progression was 5.7 months with enzalutamide versus 5.6 months with placebo (HR 0.62, 95%CI 0.53-0.74; p<0.0001). At week 13, progression of pain was significantly less common with enzalutamide (29%) than with placebo (42%, p<0.0001).

Radium-223 -

Radium-223 dichloride is a α particle-emitting agent [61] and, as a calcium mimetic, is taken up into areas of high bone turnover, such as bone metastases [62]. Once radium-223 binds bone, α particles induce double-stranded DNA breaks, causing a local citotoxic effect [63]. To date, it is the only radionuclide that showed OS benefit in CRPC. The phase III trial ALSYMPCA randomized mCRPC patients with bone metastases and without visceral metastases to receive either radium-223 or placebo in addition to the best standard of care (**Table C**) [64], having OS as the primary endpoint. Time to first SSE and time to increase in alkaline phosphatase (ALP) were among secondary endpoints. At baseline, number of bone lesions and pain intensity were similar between the arms. OS was significantly prolonged by radium-223 and time to first SSE was also improved (median 15.6 *vs.* 9.8 months; HR 0.66, 95%CI 0.52-0.83; p<0.001). The use of external beam radiation therapy to treat bone pain and the risk of spinal cord compression were significantly reduced, while radium-223 did not significantly reduce the risk of symptomatic pathological bone fracture and the need for tumor-related surgery. Decrease in ALP ≥30% occurred in 47% with radium-223 vs. 3% with placebo (p<0.001) [36]. Radium-223 provided a delay in biochemical (ALP) progression (median 7.4 vs. 3.8 months). In the ALSYMPCA study, 55% of patients required opioids at baseline [65]. Data about pain response were not collected, however in patients without opioids at baseline the proportion of patients who received opioids during study was 36% with radium-223 versus 50% with placebo, and radium-223 significantly delayed time to opioids use (HR 0.62; 95%CI 0.46–0.85). At baseline, 41% of patients were treated with BTT, and radium-223 increased OS regardless of bisphosphonate use. Delay in SSEs with radium-223 was reported both in patients not treated with BTT (although not statistically significant: median 11.8 vs. 8.4 months; HR 0.77; p=0.07) and in patients treated with bisphosphonates (median 19.6 vs. 10.2 months; HR 0.49; p=0.00048). In 2015, a systematic review evaluated the efficacy of

radiopharmaceuticals (89-strontium-chloride, 153-samarium-EDTMP, 186-rhenium-HEDP, 188-rhenium-HEDP and 223-radium-chloride) for palliation of bone pain from prostate cancer [66]. Pain response rates greater than 50%–60% were observed with all radionuclides. However, this review did not identify which radionuclide provides the best level and duration of pain relief, and OS results are not easily interpreted, because most studies were underpowered.

Bone-targeted agents

Zoledronic acid and other bisphosphonates–

Bisphosphonates reduce excessive bone turnover while preserving bone structure and mineralization. In early 1990s, several trials were initiated to investigate the use of bisphosfonates in prostate cancer: PR04 trial investigated the efficacy of sodium clodronate in locally advanced PC with negative bone scan, while PR05 investigated the same compound in bone metastatic hormone sensitive patient [67, 68]. Both trials resulted negative in terms of bone metastases- free survival and symptomatic bone PFS advantage, respectively. Mature data about OS, that was secondary endpoint, were published later: these data showed a benefit in OS only in PR05 patients, not in PR04 patients [69].

The first agent approved for the management of bone metastases in CRPC patients was zoledronic acid (ZA), a third-generation bisphosphonate. A phase III trial compared ZA versus placebo, demonstrating a significant reduction in the incidence of at least one SRE during the 24-month study period (**Table C**) [31,70]. Proportion of patients with at least one SRE was 49% with placebo and 38% with ZA (p=0.028). Furthermore, ZA significantly prolonged time to first SRE (HR 0.67; p=0.009), and time to first and subsequent SRE (HR 0.64, p= 0.002). The annual SRE incidence was 0.77 with ZA versus 1.47 with placebo (p=0.005) [71]. Pain scores and use of analgesics favored ZA. There were no differences either in disease progression or in OS [31,70]. In this study, 70% of patients treated with ZA had normalization within 1 month of the urinary levels of N-telopeptide (NTX), a markers of bone resorption. The normalization of NTX levels within 3 months correlated with a 59% reduction in the risk of death (p<0.0001) [71].

The TRAPEZE trial investigated the efficacy of addition of ZA and/or strontium-89 to docetaxel in CRPC patients [72]. Patients were randomized to receive docetaxel plus prednisolone: alone; with ZA; with a single dose of Sr89 after cycle 6 or both. Sr89

improved clinical progression free survival (CPFS), but not OS. ZA did not improve CPFS or OS but did significantly improve median SRE-free interval, mostly post-progression, suggesting a role as post-chemotherapy maintenance therapy. (**Table C**)

Of note, several trials have tested the role of zoledronic acid in patients with “earlier” phase of disease. The CALGB90202 study randomized castration-sensitive prostate cancer patients to ZA or placebo, with the aim of detecting a reduction in the risk of first SRE ~~(Table C)~~ [73]. Unfortunately, the primary endpoint was not met. Early treatment with ZA was not associated with a decreased SRE risk, compared with treatment initiation after progression to castration-resistant disease. Similarly, in the abovementioned STAMPEDE trial ~~(Table B)~~, the addition of ZA to ADT in hormone-naïve patients did not translate into a significant benefit in time to first SSE, both in the entire population and in the subgroup of patients with bone metastases [44]. On the contrary, the arm testing the addition of both docetaxel and ZA to ADT produced a significant benefit, but similar to the benefit obtained with docetaxel alone.

The ZEUS study investigated the efficacy of ZA for the prevention of bone metastasis in high-risk non-metastatic prostate cancer patients receiving ADT [74]: there was no difference in the occurrence of bone metastasis. After a median follow-up of 4.8 years, the proportion of bone metastasis was 14.7% with ZA and 13.2% in control group (p=0.65).

In conclusion, data about a *post hoc* analysis of RADAR trial, conducted in patients with locally advanced prostate cancer, must be mentioned but regarded cautiously [75]. RADAR trial investigated whether 18 months of androgen suppression (intermediate-term androgen suppression, ITAS) plus radiotherapy with or without 18 months of ZA is more effective than 6 months of neoadjuvant androgen suppression (short-term androgen suppression, STAS) plus radiotherapy with or without ZA. Secondary endpoint data and *post hoc* analyses showed that ITAS plus ZA reduce PSA progression and decrease need for secondary therapeutic intervention, in patients with Gleason 8-10 tumors. However,

neither prostate cancer-specific mortality nor all-cause mortality differed between control and experimental groups.

Considering this negative evidence in castration-sensitive and high-risk non metastatic prostate cancer patients, CRPC is the only setting of disease with proven efficacy of ZA in the management of bone metastases.

Denosumab -

Denosumab is a fully human monoclonal antibody against RANKL, and prevents the activation of its receptor, RANK, thus inhibiting osteoclast formation, function and survival, decreasing bone resorption and increasing bone mass and strength [32]. In a phase III trial that compared denosumab versus ZA in patients with bone metastatic CRPC, denosumab produced a 3.6 months significant improvement in median time to first SRE [32] (**Table C**). Furthermore, denosumab significantly delayed time to first and subsequent SREs (rate ratio 0.82, p=0.008). The two groups had a similar OS and time-to-disease progression. At week 13, median decrease in concentration of urinary N-telopeptide adjusted for creatinine (uNTX/Cr) and serum bone ALP were significantly greater with denosumab [32]. An exploratory analysis showed that, compared with ZA, denosumab significantly reduced also the risk of first SSE (HR 0.78, p=0.005) and first and subsequent SSEs (rate ratio 0.78, p=0.004) [76].

Of note, similarly to ZA, denosumab has subsequently been also tested in non-metastatic patients to evaluate its efficacy in delaying time to bone metastases. In a phase III, placebo- controlled trial in non-metastatic CRPC patients at high risk for bone metastasis, denosumab generated a 4.2- month improvement in median bone metastasis-free survival (BMFS, HR 0.85, p=0.028), in contrast with above mentioned ZEUS trial results that, however, were obtained in hormone sensitive patients [77]. Denosumab also produced a 33% reduction in the risk of symptomatic bone metastasis. However, there was no impact

on time to overall prostate cancer progression or OS (Table C) [77]. The relationship between both PSA value and PSA doubling time (PSADT) at baseline with BMFS was explored [78]. In the placebo group, patients with PSADT < 8 months had a shorter BMFS. Denosumab consistently increased BMFS among men with PSADT ≤10 months (HR 0.84; p=0.042), ≤6 months (HR 0.77; p=0.006) and ≤4 months (HR 0.71; p=0.004) [78].

Based on these results, beyond its efficacy in metastatic CRPC, denosumab has also shown a role in prolonging BMFS in high-risk non metastatic patients.

New drugs

Several new drugs have been recently or are currently being tested in prostate cancer patients. Here we summarize the results reported in studies investigating cabozantinib, dasatinib, anti-endothelin drugs, cathepsin K inhibitors and aflibercept, with specific details about bone disease control, although all these drugs did not show any improvement of survival benefit in phase III studies.

Cabozantinib -

Cabozantinib is an oral tyrosine kinase inhibitor that blocks MET, vascular endothelial growth factor receptor 2 (VEGFR-2) as well as other tyrosine kinases including RET, KIT, AXL and FLT3 [79]. MET is overexpressed in bone metastases from solid tumours, such as prostate cancer, and is involved in proliferation, differentiation and migration of osteoblasts and osteoclasts [80]. In a phase II randomized discontinuation trial, cabozantinib produced a relevant PFS prolongation compared with placebo [81]. Of note, cabozantinib showed a partial or complete resolution of bone lesions in 56% and 19% of patients and 64% of patients who received analgesics experienced an improvement in pain intensity, while 46% stopped or reduced narcotics. Similarly, in a non-randomized phase II trial, cabozantinib produced pain palliation and pain relief in 42% and 57% of patients respectively [82]. Disappointingly, two phase III randomized trials produced negative results (**Table C**) [83,84]. In the COMET-1 trial, that compared cabozantinib versus prednisone in men with progressive mCRPC pre-treated with docetaxel, abiraterone and/or enzalutamide, cabozantinib improved PFS and bone scan response, but no OS improvement was observed [83]. In the COMET-2 trial, cabozantinib was compared versus mitoxantrone in men with progressive mCRPC, and the primary endpoint of pain palliation was not met [84].

Dasatinib -

SRC, a non-receptor protein tyrosine kinase, is a key signalling molecule in tumorigenesis and bone metabolism [85]. SRC signalling has a central role in tumour growth, invasion, metastasis, and is a mediator of osteoclast activity and function, involved in pathogenesis of prostate carcinoma bone metastases [86]. Dasatinib is a potent oral inhibitor of several tyrosine kinases including SRC, SFKs members and BCR-ABL [87]. In a phase I/II trial, dasatinib was evaluated in combination with docetaxel in chemotherapy-naïve or docetaxel pre-treated mCRPC patients [88]. Fourteen patients (30%) had disappearance of at least one bone lesion and 19 patients (41%) had stable bone scans. Most of the patients had decrease in urinary NTX and BALP (87% and 76%, respectively). In a phase II trial, conducted in mCRPC chemotherapy-naive patients [89], dasatinib showed again a significant reduction of urinary NTX and ALP. In the randomized phase III READY trial, dasatinib plus docetaxel was compared to docetaxel plus placebo in mCRPC chemotherapy-naive patients (**Table C**) [90], with OS as primary endpoint and SREs and pain palliation as secondary end points. Dasatinib failed to improve OS, while median time to first SRE was 31.1 months with placebo and not reached with dasatinib (HR 0.81, p=0.08). Reduction in pain intensity was not significantly different between arms.

Anti-endothelin -

Endothelins (ET-1, ET-2 and ET-3) are a family of small peptides with multiple roles including regulation of the vasomotor tone, nociception, hormone production and cellular proliferation [91]. ET-1 stimulates osteoblast activity and plays a key role in promoting prostate cancer growth and metastasis [92]. The activity of ET-1 is mediated by endothelin A receptor (ET-A) [93]. In preclinical models, endothelin receptor antagonists showed inhibition of the development and progression of metastases [94].

Atrasentan is a potent, oral, selective ET-A antagonist that inhibits the osteoblast-dependent formation of new bone induced by metastatic cancer cells [95]. In a phase II, placebo-controlled trial, atrasentan was tested in hormone refractory metastatic prostate cancer (HRPC) patients [96]. The primary endpoint was the rate of pain relief after 12 weeks, that was not met. However, atrasentan 10 mg produced a statistically significant improvement in BPI, particularly the benefit was demostrated in pain interference with relations with other people (p=0.031) and in the worst pain in the last 24 hours (p=0.03). In another phase II trial in asymptomatic HRPC patients [94], markers of bone deposition and resorption were significantly reduced with atrasentan compared to placebo. A phase III randomized, placebo-controlled trial evaluating atrasentan in non-metastatic HRPC (**Table C**) did not meet the primary endpoint of delaying time to disease progression and did not show a significant improvement in time to first skeletal lesion, although atrasentan lengthened PSA doubling time (p=0.031) and slowed BALP increase (p<0.001) [97].

Zibotentan is an oral, selective ET-A antagonist, competing with ET-1 for receptor binding and therefore indirectly increasing pro-apoptotic signalling. Three trials (ENTHUSE) evaluated zibotentan in CRPC patients (**Table C**) [98-100]. Disappointingly, in these trials there was no significant improvement either in OS, the primary endpoint, or in secondary endpoints, including time to pain progression and pain response.

Cathepsin K inhibitors

Cathepsin K is a cysteine protease, expressed in osteoclasts and various type of cancers [101]. It plays a key role in osteoclast-mediated bone resorption and promotes tumor cells invasion [102]. Cathepsin K inhibitors have been studied for post-menopausal osteoporosis and bone metastatic disease [103]. Odanacatib, a cathepsin K inhibitor, has been evaluated in a randomized, double blind trial in order to assess the efficacy and safety in reducing markers of bone resorption in bone metastatic breast cancer patients

[104]. Forty-three patients were randomized to oral odanacatib 5 mg daily for 4 weeks or intravenous ZA 4 mg given once at study initiation. The study showed that odanacatib reduced uNTx similarly to ZA after 4 weeks of treatment [104].Two phase III clinical trials were planned in order to evaluate its efficacy and safety in prolonging time to first bone metastasis in CRPC patients (NCT00691899) and in reducing risk of bone metastases in women with breast cancer (NCT00692458). Unfortunately, these studies were closed before starting accrual [105]. Further clinical trials are needed in order to obtain more clinical informations.

Aflibercept

Aflibercept is an anti-angiogenic agent with high affinity to the isoform VEGF-A, it also binds VEGF-B and platelet-derived growth factors PlGF1 and PlGF2 [106]. A recent phase III, randomized, double-blind placebo-controlled trial (VENICE) has evaluated docetaxel plus aflibercept *vs* docetaxel plus placebo in 1224 mCRPC patients [107]. The primary endpoint was OS; secondary endpoints included PFS, PSA-PFS, time to first SRE and pain-PFS. Aflibercept has not met its primary endpoint (22.1 months *vs* 21.1 months; p=0.38). There were not differences in terms of secondary end-points, in particular median time to first SRE was 15.3 months in aflibercept group *vs* 15.0 months in placebo group (p=0.31).

CONCLUSIONS

Recently, a better understanding of mechanisms associated with bone metastatic disease in prostate cancer and, more specifically, the crucial role of cross-talk between tumor cells and bone micro-environment in metastatic progression provided the basis for the development of new effective bone-targeted therapies.

There is no question that prostate cancer cells have a strong bone tropism, and their dissemination into the bone alters the equilibrium between osteoclasts and osteoblasts. Although bone lesions secondary to prostate carcinoma are mainly characterized by aberrant osteoblast activation, osteolysis is common and is responsible of increased incidence of SREs that are dramatic clinical events, able to decrease QoL, autonomy and survival of CRPC patients.

Abnormal osteoclast activity is the rationale for the administration of potent osteolysis inhibitors, such as zoledronic acid and denosumab. These agents reduce the burden of bone metastatic disease, although this benefit does not translate in an improvement in survival.

Recently, a new treatment opportunity for patients with prostate cancer and bone metastases is represented by radium-223. Notably, this α-emitter, when used in men with CRPC and bone metastases, not only showed efficacy in preventing symptomatic skeletal events, but it was the first bone-targeted therapy associated with a significant OS improvement.

Additionally, in the last five years, highly effective new systemic agents have significantly changed the treatment landscape of CRPC patients, improving their life expectancy [12]. Some of these therapies also documented efficacy in delaying SRE and improving bone pain. Trials testing the concomitant administration of radium-223 with abiraterone (NCT02043678), enzalutamide (NCT02194842) and docetaxel (NCT01106352) are ongoing. Results of these studies will help to better understand how to combine systemic

new agents with bone-targeted therapies, in order to effectively interfere with the “seed” and with the “soil” at the same time.

Figure legends

Figure A. Mechanisms of shift from hematopoietic stem cell niche to “onco-niche”.

A) Hematopoietic stem cell into the bone marrow is localized in the hematopoietic stem cell niche in connection with osteoblasts through SDF-1/CXCR4 pathway. Prostatic tumor cells are able to compete with hematopoietic stem cell for the place in the bone marrow using SDF-1/CXCR4 axis, favoring the formation of “onco-niche.

B) In the “onco-niche”, cancer cell may remain in a state of dormancy or may start to colonize and invade bone.

Figure B. Pathogenesis of “vicious cycle” that underpin osteoblastic bone metastases from prostate carcinoma.

A) In the early phase of metastatic colonization osteolysis predominates due to production of transforming growth factor β1 (TGF β1), parathyroid-hormone-related peptide (PTHrP) and interleukin 6 (IL-6). These factors activate the receptor activator nuclear kappa B (RANK)/RANK ligand (RANKL) pathway, which is responsible of bone resorption stimulation.

B) The increase of osteolysis causes the release from bone matrix of growth factors and cytokines responsible for neoplastic proliferation.

C) In the next phase of skeletal colonization bone neoapposition become dominant due to growth factors released by cancer cells and from bone matrix, such as basic fibroblast growth factor (bFGF), bone morphogenic proteins (BMPs), endothelin-1 (ET-1), tumour growth factor b1 (TGFb1) and insulin-like growth factor 1 (IGF-1), able to stimulate osteoblasts activity.

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